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Expression of insulin-like growth factor-I and -II genes in rat medullary thyroid carcinoma

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Several types of cancer cells produce polypeptide growth factors and often the same cells have functional receptors for the released growth factor (autocrine secretion). We have studied expression of genes encoding somatomedin-C/insulin-like growth factor-I (Sm-C/IGF-I) and IGF-II, in rat medullary thyroid carcinomas (MTCs) in different stages of tumour differentiation. RNAs hybridizing specifically to an IGF-I cDNA probe were detected in 6 out of 7 differentiated MTCs and IGF-II related RNAs were demonstrated in 5 out of these 7 differentiated MTCs. In 5 anaplastic MTCs no IGF RNAs were detected, except for a small amount of IGF-II related RNA in one tumour.

Insulin-like growth factor; Gene expression; Northern blotting; Tumor differentiation; (Rat medullary thyroid carcinoma)

1. INTRODUCTION

'Autocrine secretion' by cancer cells has been demonstrated for transforming growth factors α and β, platelet derived growth factor and bombesin [1–3]. This mechanism enables these cells to establish autonomous growth without the need for 'external' growth factors.

An autocrine system involving somatomedin-C/insulin-like growth factor-I (Sm-C/IGF-I) has been suggested for human osteosarcoma cell lines, which were shown to produce immunoreactive IGF-I [4]. Because of the high degree of nucleotide sequence conservation between human and rodent IGF-genes, the availability of cDNA probes encoding human IGF-I [5] and IGF-II [6], allowed us to study expression of these genes in rat tissues.

Medullary thyroid carcinoma (MTC) is a tumour originating from the calcitonin (CT) producing C-cells of the thyroid gland [7]. Rats of the Wag/Rij strain spontaneously develop these tumours. After sequential transplantations some of these tumours become anaplastic. They show an increased growth rate and secrete smaller amounts of CT [8]. Using Northern blot hybridization analysis, we examined the presence of RNAs encoding IGF-I, IGF-II and CT in both types of rat MTC.

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2. MATERIALS AND METHODS

2.1. *RNA isolation and Northern blotting*

Total cellular RNA was isolated from rat MTCs and adult rat liver, by centrifugation of a tissue

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homogenate through a cesium chloride cushion [9]. Poly(A)-enriched RNA was prepared by one cycle of oligo(dT)-cellulose column chromatography [10]. RNA preparations were denatured with glyoxal, size-fractionated electrophoretically on 1.4% agarose gels, transferred onto Gene Screen membranes by Northern blotting [11], and hybridized to ^{32}P-labelled probes.

2.2. *DNA probes and hybridization*

As an IGF-I specific probe we have used plasmid pIGF-I [5], containing a 777 base pairs (bp) cDNA sequence, encoding the complete amino acid sequence of human IGF-I. The extent of nucleotide sequence homology between human and rat IGF-I RNA is not exactly known. However, since human IGF-I probes show cross-hybridization with Chinese hamster [12] and mouse [13] genomic DNA, we assumed that the human IGF-I cDNA could be used as a specific probe for rat IGF-I RNA. In addition, an extensive conservation of the amino acid sequence in the N-terminal parts of rat and human IGF-I has been demonstrated [14].

As an IGF-II specific probe we have used plasmid pIGF-II var [6], containing a 713 bp cDNA sequence, encoding the complete amino acid sequence of a variant human IGF-II. Since the nucleotide sequence homology between the coding regions of human IGF-II var and rat IGF-II/multiplication stimulating activity (MSA) cDNA is 84% [6,15], our human IGF-II var cDNA was considered to be a suitable probe for detecting rat IGF-II/MSA related RNAs. Previous experiments have shown that the IGF-I and IGF-II cDNA probes do not cross-hybridize under the conditions as employed in the present study. In addition, the synthetic 20-mer oligonucleotide 5′-ACTGAAGCGTGTCAACAAGC-3′ complementary to nucleotides 1171–1190 of the rat mRNA encoding MSA [15] was used as a probe.

As a CT-specific probe we have used plasmid pCal [16], containing a 627 bp long insert of rat CT cDNA (obtained from Dr M.G. Rosenfeld).

Double-stranded DNA probes were labelled with ^{32}P by nick-translation to a specific activity of 10^8 dpm per μg DNA. The MSA-specific oligonucleotide was labelled 5′-terminally with [γ-^{32}P]ATP and T_4-polynucleotide kinase. Hybridizations were performed in the presence of 50% formamide and 6 × standard saline citrate (SSC) at 42°C (30°C for the oligonucleotide) for 18 h. Filters were washed twice at 65°C (32°C for the oligonucleotide) in 2 × SSC–0.5% SDS for 30 min, and exposed to Fuji RX films.

The sizes of RNA molecules hybridizing to the probes were determined by coelectrophoresis of molecular mass standards (*Hin*dIII-digested λDNA and *Hae*III-digested ϕX 174 DNA).

3. RESULTS AND DISCUSSION

The results have been summarized in table 1.

In 6 out of 7 differentiated MTCs examined, hybridization of the IGF-I specific probe to an RNA species of approx. 7000 nucleotides was observed. In addition, IGF-I related RNAs of 2000 and 1000 nucleotides were detected in 5 and 2 of these tumours, respectively. The 7000 nucleotides long RNA species could also be demonstrated in adult rat liver, where IGF-I is known to be synthesized (fig.1A). Since the hybridization signal is much stronger in the poly(A)-enriched RNA preparations from differentiated MTCs and liver than in the total cellular RNA preparations, we conclude that the hybridizing RNAs are polyadenylated. Whether the differently sized RNA species are transcripts of different rat IGF-I genes or represent alternatively processed

Table 1

Presence and size (in nucleotides) of IGF-I, IGF-II and CT related RNA species in rat MTCs and rat liver, as revealed by Northern blot hybridization analysis

Probe	Tissue		
	Differentiated MTC	Anaplastic MTC	Adult liver
IGF-I	7000 (6/7)[a]		7000
	2000 (5/7)	–[b] (5/5)	1700
	1000 (2/7)		1300
			600
IGF-II var	3800–4300 (5/7)	2800 (1/5)	–
CT	3000 (7/7)		
	1050 (7/7)	– (5/5)	–

[a] Number of tumours in which the specific RNA species was detected/total number of tumours of that type investigated

[b] –, not detected

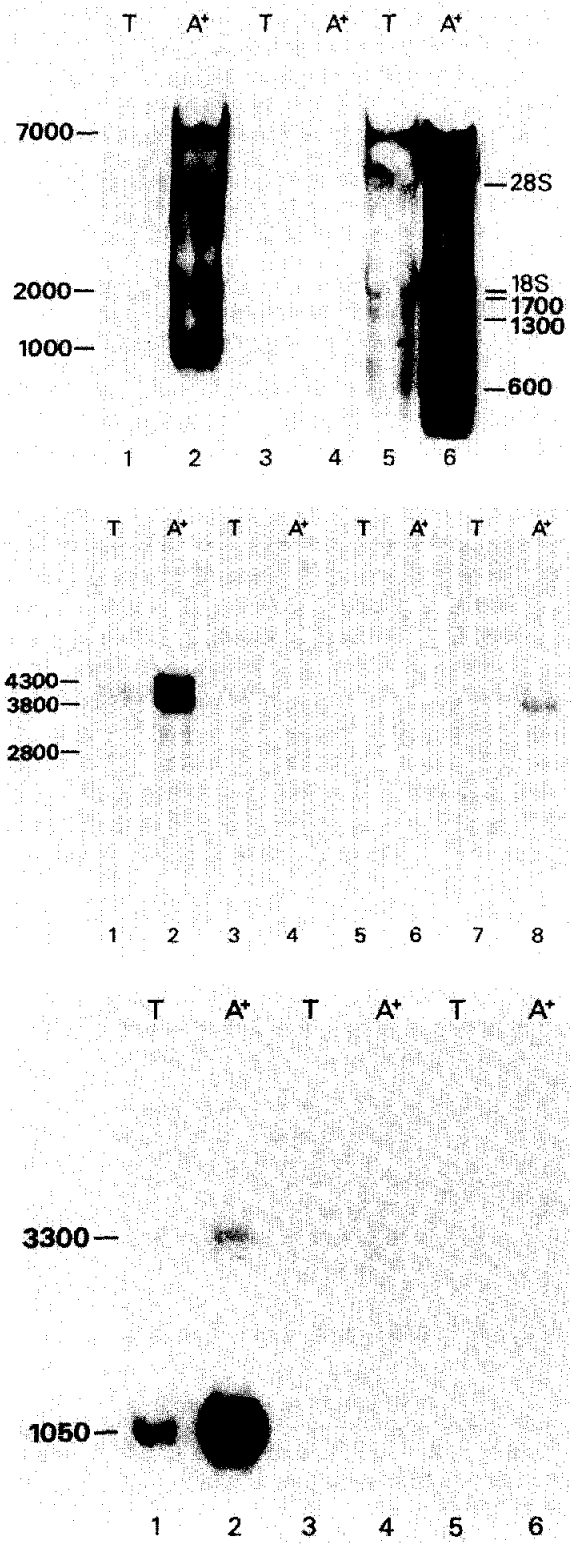

Fig.1. Detection of IGF-I, IGF-II and calcitonin related transcripts in RNA preparations from rat MTCs and rat liver. Total cellular (T) and poly(A)-enriched (A^+) RNA preparations (20 μg per lane) include those from (lanes): a differentiated rat MTC (1, 2, 7 and 8); an anaplastic rat MTC (3 and 4); an adult rat liver (5 and 6). The size (in nucleotides) of hybridizing RNA species and the positions of 28 S and 18 S ribosomal RNAs are indicated. (A) Hybridization with the 777 base pairs (bp) *Pst*I insert of plasmid pIGF-I. The blot was exposed for 13 days with an intensifying screen. Lanes 5 and 6 were exposed for 2 days with an intensifying screen. (B) Hybridization with the 713 bp *Pst*I insert of plasmid pIGF-II var. Lanes 1 and 2 were exposed for 18 h with an intensifying screen, lanes 3–8 were exposed for 3 days with an intensifying screen. The 2800 nucleotides long IGF-II related RNA can hardly be seen in lane 4, but it was clearly visible on the original autoradiographs. (C) Hybridization with the plasmid pCal, containing a 627 bp insert of rat CT cDNA. Lanes 1 and 2 were exposed for 2 h with an intensifying screen, lanes 3–6 were exposed for 3 days with an intensifying screen.

transcripts of a single gene is not known. We and others [12,13,17,18] have shown that the human genome contains a single IGF-I gene, which is located on chromosome 12. However, this does not preclude the occurrence of multiple genes in other species.

IGF-II related poly(A) RNAs were demonstrated in 5 out of 7 differentiated MTCs, but not in adult rat liver (fig.1B). In differentiated MTCs four species of IGF-II RNA are found in the range of 3800–4300 nucleotides.

In 5 anaplastic rat MTCs no IGF-I encoding RNAs could be detected. However, an IGF-II related RNA was demonstrated in 1 out of these 5 tumours. Interestingly, the size of the IGF-II RNA molecules is dissimilar for the two types of MTC. In the anaplastic MTC the IGF-II RNA is approx. 2800 nucleotides long. Hybridization of RNA from differentiated rat MTCs to the rat MSA specific oligonucleotide, revealed four RNA species with the same length as detected by the human IGF-II cDNA probe (fig.1B). In RNA preparations of the IGF-II positive anaplastic rat MTC, the MSA-specific oligonucleotide did not reveal the 2800 nucleotides long RNA species detected with the human cDNA probe, probably because of its low abundancy. We and others

[13,18,19] have shown that in humans there is only a single IGF-II gene, which is located on chromosome 11. However, two closely related types of cDNA molecules, encoding precursors of IGF-II and a variant of IGF-II (IGF-II var) have been isolated from the same human liver cDNA library [6]. The presence of different RNA species hybridizing to an IGF-II specific probe in rat MTC tissue, might be explained by alternative splicing or usage of different promoter and/or polyadenylation signals. On the other hand, it might point to the existence of related but as yet unknown rat IGF-II genes. In this respect it is important to note that both developmental and tissue-specific expression of several rat pre-pro-IGF-II related transcripts have been reported [20].

Differentiated as well as anaplastic rat MTCs have been reported to contain the polypeptide hormone CT [8]. However, we were able to demonstrate the presence of CT-encoding mRNA in differentiated MTCs only (fig.1C, table 1). This indicates that CT mRNA production in anaplastic MTCs is below the level of detection on Northern blots and might be used as a biochemical marker for distinguishing differentiated MTC from the anaplastic type.

The presence of IGF-like peptides in normal rat thyroid glands has not yet been reported. IGF-I was undetectable by immunoperoxidase staining of thyroid tissue sections from man and growth hormone-treated Little mouse [21]. Therefore the question remains open whether the rat MTC tumours might originate from cells that produce IGFs in the normal thyroid gland, or that IGF RNA production is a newly acquired characteristic of the transformed MTC cells.

If the rat MTCs are capable of producing IGFs, it will be interesting to study whether the IGF-producing cells have functional receptors for these polypeptide growth factors. If so, autocrine secretion of IGFs by rat MTCs might prove to be a useful model for physiological experiments studying functional relations between IGF production and tumourigenesis or tumour progression.

It was recently demonstrated that human breast cancer cells in culture secrete an IGF-I-like protein and that these cells were growth stimulated by serum-derived IGF-I [22]. It was already known that several human breast cancer cell lines have cell surface receptors for IGF-I [23]. These results suggest that IGF-I can be involved in an autocrine system of cell proliferation in breast cancer cells.

The detection of IGF-I related RNAs in differentiated rat MTCs represents one of the first demonstrations of IGF-I RNA in tumour tissue. IGF-II RNA has recently been demonstrated in human Wilms tumours, rhabdomyosarcomas and various normal tissues [24,25].

The fact that IGF-I RNA production can be detected (although in small amounts) in the majority of tumours of the differentiated type of rat MTC but not in the anaplastic type, might be an important clue to the molecular and cellular basis of tumour differentiation and malignancy and perhaps even differentiation in general, since IGF-I synthesis and IGF-II synthesis are under different control and they presumably have different biological functions in vivo [26]. In particular, IGF-II (MSA) might have a more pronounced role in the fetal organism, while Sm-C/IGF-I is the predominant somatomedin postnatally.

As described by others, IGF-II RNA can easily be detected in a variety of fetal and neonatal rat and human tissues, but can hardly be detected in adult rat, mouse and human liver [20,25], even though the liver is considered to be one of the major sites of IGF-II production in adult organisms [25]. IGF-II RNA was undetectable in adult rat liver in our experiments. These results suggest that the relatively large amount of IGF-II RNA detected in differentiated rat MTCs reflects a tumour-specific activation or enhanced expression of (an) IGF-II gene(s) in this type of neoplasm.

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